ABSTRACT OF THE DISCLOSURE

Methods and compositions for producing single-stranded cDNA (ss-cDNA) with a vector-based system in eukaryotic cells. In one embodiment, the vector comprises plasmid(s) that contain a reverse transcriptase/RNAse H gene and a cassette, the cassette including a sequence of interest having an enzymatic sequence included therein, an inverted repeat, and a primer binding site, which produces an RNA template from which the reverse transcriptase synthesizes ss-cDNA of a specified sequence. The ss-cDNA is then modified to remove flanking vector sequences by taking advantage of the "stem-loop" structure of the ss-cDNA, which forms as a result of the inclusion of an inverted tandem repeat that allows the ss-cDNA to fold back on itself, forming a double stranded DNA stem with the sequence of interest in the loop portion of this intermediate. The double-stranded stem may also contain one or more restriction endonuclease recognition sites and the double-stranded stem of the stem-loop intermediate is cleaved by the desired corresponding restriction endonuclease(s) so that the loop portion, or sequence of interest and sequence with enzymatic activity, is then released as a linearized, single-stranded piece of DNA. The plasmid may also include a gene for producing the restriction endonuclease specific for this site in the stem. This released ss-DNA sequence contains minimal sequence information either upstream 5' or downstream 3' from the previous double stranded stem portion which contains the restriction endonuclease cut site. The plasmid may also include a second sequence of interest 3' to the inverted repeats which is likewise produced with minimal vector sequence.

In a second aspect, the components utilized to produce ss-DNA *in vivo* in accordance with the present invention include a reverse transcriptase gene, a sequence of interest located between inverted repeats or 3' to the inverted repeats, and a primer binding site located 3' to the cassette including the sequence of interest and the inverted repeats. The sequence of interest includes a sequence having enzymatic activity when single stranded. The invention also contemplates a second sequence of interest located between the inverted repeat and the primer binding site, and the functions and signaling instructions for transcription of these components *in vivo*.